C-Reactive Protein Colocalizes With Complement in Human Hearts During Acute Myocardial Infarction

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Background Rises in circulating C-reactive protein (CRP), the prototypical acute-phase protein in humans, correlate with clinical outcome in patients with myocardial ischemia and infarction. We hypothesized that these correlations might reflect active participation of CRP in the local inflammatory response ensuing in the jeopardized myocardium because on binding to a ligand, CRP is able to activate the classic pathway of complement, and in addition, complement activation has been shown to occur locally in infarcted myocardium.

Methods and Results To verify our hypothesis, we investigated localization of CRP in relation to deposition of complement in tissue specimens of infarcted and healthy heart tissue obtained from 17 patients who had died after acute myocardial infarction. CRP was found to be deposited only in infarcted regions and not in normal-appearing areas of the myocardium, being colocalized with depositions of C4 and C3 activation fragments of the complement system. Deposition of CRP and complement in infarcted myocardium appeared to be time dependent, because it was found in all infarctions except for one of young age (<12 hours old) and two of greater age (>1 year old), whereas another tissue specimen of an infarct <12 hours old showed only moderate but positive staining for both CRP and complement in comparison with older infarctions.

Conclusions We conclude that in humans, CRP may localize in infarcted heart tissue and suggest that this acute-phase protein promotes local complement activation, and hence tissue damage, in acute myocardial infarction. (***Circulation.* 1997;95: 97-103.**)

Key Words • proteins • myocardial infarction • immunohistochemistry • immunology

Impaired perfusion of the myocardium results in a local inflammatory response[1,2] that comprises a complicated interaction between ischemic myocardial and inflammatory cells, cytokines, complement factors, and acute-phase proteins. The impact of inflammation on the extent of tissue damage after myocardial ischemia and infarction in humans is not clear. However, studies in animals have shown that local inflammatory reactions may contribute significantly (up to 65%) to infarct size.[1-3]

Inherent to the inflammatory process is the occurrence of an acute-phase response.[4-6] This response is induced by proinflammatory cytokines, which are released from the inflamed tissue by inflammatory and/or parenchymal cells[7,8] and stimulate the liver to synthesize a number of acute-phase proteins.[9] CRP is regarded as the prototype of acute-phase proteins in humans. Circulating levels of CRP have been shown to increase in patients suffering from AMI.[5,6] The physiological role of CRP is not fully understood; it was discovered by its ability to bind to pneumococcal C-polysaccharide,[10] but various other anti-inflammatory and proinflammatory functions of CRP have been described as well.[11-15] One of the proinflammatory properties observed in vitro is the ability of CRP to activate complement via the classic pathway.[13-15] This has led to the hypothesis that after tissue injury, CRP binds to damaged cells and, by activating complement, may contribute to the inflammatory response.[13-16] In agreement with this, CRP has been found to be localized in inflamed tissues,[17,18] including infarcted myocardium in rabbits.[19,20] In clinical studies, circulating levels of CRP were found to correlate with total infarct size in AMI[6] and with prognosis in unstable angina.[21] Yet, direct evidence that CRP participates in the local inflammatory response of human AMI is lacking, although local complement activation has been shown to occur in this condition.[22,23]

To examine a possible role of CRP in the local inflammatory reactions ensuing in infarcted human myocardium, we investigated the localization of CRP in relation to deposition of activated complement in infarcted human heart tissue. To this end, immunohistochemical studies were performed using tissue specimens obtained from 17 patients who died after AMI.

Methods

Patients

All patients included in this study had recently died and were referred to the department of pathology for autopsy within 24 hours after death. Patients were included in the study when at autopsy they showed signs of a recently developed AMI, ie, de-

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Selected Abbreviations and Acronyms

AMI = acute myocardial infarction
CRP = C-reactive protein
DAB = 3,3′-diaminobenzidine tetrahydrochloride
LDH = lactate dehydrogenase
MAb = monoclonal antibody
PMN = polymorphonuclear leukocyte

creased LDH staining (decoloration) of the affected myocardium on histochemical examination. Patients did not necessarily have clinical evidence of AMI shortly before death, that is, specific ECG criteria or a rise in cardiac enzymes (serum creatine kinase and serum creatine kinase MB fraction). The study protocol was approved by the ethics committee of the Free University Hospital Amsterdam.

Processing of Tissue Specimens

Specimens were obtained from the infarcted myocardium as well as from adjacent sites. The latter sites showed normal LDH staining and were studied as internal controls. Tissue specimens were stored at −196°C (in liquid nitrogen) before being prepared as frozen sections. The glass slides used for microscopy were pretreated with 0.1% poly-L-lysine (Sigma Chemical Co) to enhance adherence of the frozen tissue sections.

Antibodies

An MAb (MAb 5G4) against CRP was obtained by fusing spleen cells from a mouse immunized with human CRP with SP2/0 cells as described by Wolbink and colleagues.[24] The MAbs against complement factors used in this study have been described[25,26] and are summarized in Table 1. MAbs were purified from hybridoma culture supernatant or ascitic fluid by protein G or A chromatography (Pharmacia Fine Chemicals) and stored at 1 mg/mL in PBS.

Immunohistochemistry

Frozen sections of 4 μm were mounted onto glass slides, dried for 1 hour by exposure to air, and fixed in acetone (Baker Analyzed Reagent, Mallinckrodt Baker BV). After a rinse in PBS, they were incubated with normal rabbit serum (Dakopatts A/S) diluted 1 to 50 in PBS containing 1% wt/vol BSA (PBS-BSA) (BSA from Boehringer Mannheim GmbH) at room temperature for 10 minutes. The slides were then incubated for 60 minutes with specific antibody solutions (each MAb at a 1 to 5000 dilution in 1% wt/vol PBS-BSA, except for MAbs C4-1 and 5G4, which were diluted 1 to 500). In control experiments, similar incubations were performed with two irrelevant mouse monoclonal antibodies: IgG1 (anti–phospholipase A_2, kindly provided by Dr F.B. Taylor, Jr, Oklahoma Medical Research Foundation, Oklahoma City) and mouse myeloma protein, MOPC 21 (Cappel, Organon Teknika) as well as with an irrelevant IgG2a MAb (against placental alkaline phosphatase). The slides were washed for 30 minutes with PBS and incubated with horseradish peroxidase–conjugated rabbit anti-mouse immunoglobulins (RaM-HRP, Dakopatts) diluted 1 to 25 in 1% wt/vol PBS-BSA. Thereafter, the slides were washed again in PBS; incubated for 4 minutes in 0.5 mg/mL DAB (Sigma) in PBS, pH 7.4, containing 0.01% vol/vol H_2O_2; washed again; counterstained with hematoxylin for 40 seconds; dehydrated; cleared; and finally mounted.

To match depositions of CRP and complement in the myocardial tissue, double stainings for CRP and C3d or CRP and C4d, respectively, were performed. The anti-CRP MAb 5G4 and the complement MAbs belong to different IgG subclasses (see Table 1). Therefore, double staining was performed with goat anti-mouse IgG1 and goat anti-mouse IgG2a. Frozen sections (air-dried and acetone-fixed) were preincubated with normal goat serum (diluted 1 to 20) as described above for normal rabbit serum. The sections were then incubated with appropriate mixtures of MAbs: 5G4 diluted 1 to 50 and C4-4, 1 to 5000; or 5G4, 1 to 50 and C3-15, 1 to 5000. After a washing with PBS, the slides were incubated simultaneously with horseradish peroxidase–conjugated goat anti-mouse IgG2a and biotin-conjugated goat anti-mouse IgG1 (Southern Biotechnology Associates Inc), both diluted 1 to 50. The complement MAbs were then visualized by incubation of the slides with streptavidin-alkaline-phosphatase complex (1000 U/mL) diluted 1 to 250 (Boehringer), followed by exposure of the slides to naphthol AS-MX and fast blue BB base (both from Sigma). The CRP/5G4 MAb was demonstrated by exposure of the slides to the DAB solution as described above for the single staining procedure. For all myocardial tissue specimens, the age of the AMI was estimated by microscopic criteria,[27,28] which included intensity of eosinophilic staining of involved myofibers, loss of nuclei and cross-striation, PMN and lymphocyte infiltration, and fibrosis. All slides were judged by three independent investigators (W.K.L., H.W.M.N., and L.H.J.), each scoring for infarct age and anatomic localization of specific antibody as visualized by immunohistochemical staining. The anatomic localizations examined were the myofiber (membrane, cytoplasm, nucleus, cross-striations) and blood-vessel elements (endothelium and surrounding tissue). Distinctions were made between negative, moderate positive, and strong positive immunostaining. In addition, the presence of PMN or lymphocyte infiltration was scored for. For the final scoring results, consensus was achieved by the three investigators.

Results

Patients

Myocardial tissue specimens were obtained from 17 patients who had died after AMI, as shown by autopsy performed within 24 hours after death. Except for 4 patients (patients 4, 6, 12 [streptokinase], and 17 [coronary surgery], Table 2), none of the patients had received reperfusion therapy. Three patients (patients 1, 3, and 11) had a history of previous AMI. Most patients had a posterior wall infarction, but other locations were involved as well (Table 2). Specimens were obtained from the infarcted myocardium as well as from normal-appearing myocardial tissue. The age of the infarct, assessed by use of microscopic criteria described before,[27,28] varied from <12 hours up to >2 weeks (Table 2). From patients 1 and 11, tissue specimens were also taken from the old, fibrotic myocardial infarction sites (anamnestically 1 and 10 years old, respectively).

Localization of CRP

By immunohistochemistry, CRP was found to be localized in 16 of the 17 myocardial infarctions studied. An example of the observed staining patterns is given in Fig 1A, which shows positive CRP staining in infarcted myocardial tissue. In most infarctions, CRP was found to be localized on the jeopardized myofibers. Except for 1 section with moderate and 3 with strong positive immunostaining, CRP was not found to be located on blood vessels. The overall results and the intensity of the immunostaining for CRP are listed in Tables 3 and 4, re-

TABLE 1. Antibodies Used for Immunohistochemical Examination

MAb	Antigen	Antibody Subclass
5G4	C-reactive protein	IgG-2a
C4-4	C4d	IgG-1
C4-1	C4c	IgG-1
C3-15	C3d	IgG-1
C3-9	C3c	IgG-2a
C3-5	C3a	IgG-1

TABLE 2. Patient Characteristics

Patient	Sex	Age, y	Reason for Admittance	Cause of Death	History of AMI	Sites of Recent AMI	Reperfusion Therapy for Recent AMI	Age of Recent AMI	% Jeopardized Myofibers in HE Staining
1	M	81	AMI	AMI	++	PW	–	<12 h	–
2	M	51	AMI/pneumonia	AMI/lung emphysema	–	PW	–	<12 h	–
3	M	61	Triple arthrodesis	AMI	+	PW/LW/AW	–	12-24 h	50
4	M	60	AMI	AMI	–	PW/LW/AW/septal	+, SK	1-3 d	60
5	F	69	AMI	AMI	–	PW/RV/septal	–	1-3 d	60
6	F	58	AMI	AMI/AVS	–	AW/septal	+, SK	1-3 d	50
7	F	62	Coronary surgery	AMI, postoperative	–	AW/PW/septal	–	3-5 d	25
8	M	75	AMI	AMI	–	AW/PW/septal	–	3-5 d	65
9	M	75	AMI	AMI+tamponade	–	PW	–	3-5 d	65
10	F	54	AMI	AMI/DIC	–	PW/RV	–	5-9 d	70
11	M	63	AMI	AMI/AAAA	++	AW	–	5-9 d	20
12	M	70	AMI	AMI	–	PW/RV	+, SK	5-9 d	20
13	M	80	AMI	AMI+tamponade	–	LW	–	5-9 d	40
14	F	82	AMI/AVR	AMI	–	septal	–	5-9 d	25
15	M	63	AMI	AMI/CVA	–	LW/PW	–	9-14 d	50
16	M	66	AMI	AMI	–	AW/PW/septal	–	9-14 d	60
17	M	53	AMI due to aortic dissection type A	AMI due to aortic dissection type A	–	PW/RV/septal	+, surgery	9-14 d	80

HE indicates hematoxylin-eosin staining (in LDH-unstained sites; estimation of percentage of jeopardized myofibers by area of eosinophilia in frozen tissue sections); AVR, aortic valve replacement; AVS, aortic valve stenosis; DIC, diffuse intravascular coagulation; AAAA; acute abdominal aortic aneurysm; CVA, cerebrovascular accident; PW, posterior wall; LW, lateral wall; AW, anterior wall; RV, right ventricle; +, 1 AMI in history; ++, >1 AMI in history; and –, no AMI in history.

spectively. Remarkably, CRP was not detected in the tissue samples from one patient (patient 1) with the most recent AMI (infarct age, between 0 and 12 hours). The tissue sample from patient 2 (infarct age, also between 0 and 12 hours) showed positive staining for CRP but not as distinct as seen in the older myocardial infarctions (1 to 14 days old). In the internal control samples of patients 1 and 11, remnants of old myocardial infarction (anamnestically, 1 or 10 years old, respectively) were seen, characterized by fibrotic parts in the remaining myocardium. These sites also did not show any staining for CRP. However, in addition to a negative staining in the 10-year-old myocardial infarction site, a strong, positive staining was found for CRP in the tissue specimen taken from the recent myocardial infarction site in patient 11 (between 5 and 9 days old). In samples from early infarctions, that is, <24 hours old, the sarcolemma of the myofibers was stained more distinctly. Staining of cytoplasm of the myofibers, in addition to that of the sarcolemma, was seen most clearly in the older infarct samples (age, >24 hours). No positive staining was found for nuclear elements. Cross-striation stained for CRP in 10 of 17 of the infarct samples.

We included a number of controls to check the specificity of the observed staining for CRP. The LDH-unstained (ie, infarcted) myocardial samples contained various amounts (20% to 80%, see Table 2) of apparently normal, healthy cardiomyocytes. These normal-appearing areas in the infarct region did not show any significant staining for CRP (see, for example, Fig 1A). The internal controls, taken from the noninfarcted (ie, the LDH-stained) sites of myocardium from the same patient, also did not show any positive staining for CRP. Significant staining seemed to be independent of the presence of PMN and/or lymphocyte infiltration. In addition, a similar staining procedure performed with an irrelevant IgG2a MAb yielded negative results with all tissue specimens (data not

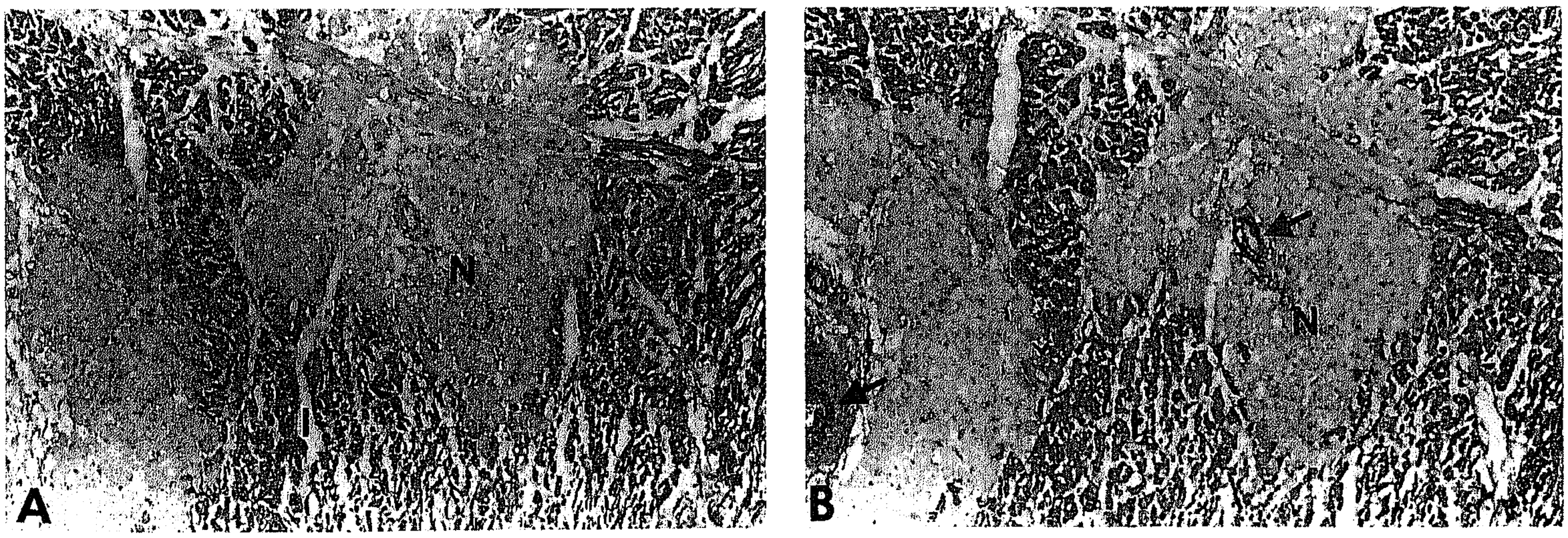

FIG 1. Immunohistochemical localization of CRP (A) and complement activation fragment C3d (B) in infarcted myocardium (I indicates infarcted and N, normal/noninfarcted myocardium) with MAbs 5G4 and C3-15, respectively, as described in "Methods" (patient 13 [Table 2], frozen tissue sections from the same myocardial infarction site). Similar staining patterns were seen for CRP and C3d in fibers of infarcted myocardium. C3d was also localized in vascular elements (arrows) (magnification ×100).

TABLE 3. Localization of CRP and Complement in Myocardial Infarction

	Anatomic Localization (No. Positive/Total)		
Antigen	Myofiber	Blood Vessel	Myofiber+Blood Vessel
CRP	16/17	4/17	4/17
C4d	15/17	12/17	11/17
C4c	11/17	5/17	5/17
C3d	16/17	15/17	15/17
C3c	13/15*	6/15*	6/15*
C3a	0/17	0/17	0/17

*Two slides neglected because of high background staining invalidating proper immunohistochemical scoring procedure.

shown). Staining for CRP of myocardial tissue specimens obtained from a patient who died of sepsis also yielded negative results, ruling out the possibility that positive staining for CRP observed with the tissue specimens of the patients with AMI was merely due to the presence of a systemic inflammatory response. Finally, myocardial tissue specimens from an immature child who died in utero at 22 weeks' duration of amenorrhea, regarded as a pure, nonischemic myocardial control, also did not show any staining for CRP.

Deposition of Complement

All tissue specimens were analyzed for the presence of activated complement fragments by use of various MAbs. Both C4d (C4-4) and C3d (C3-15) were found to be deposited in most infarctions in a pattern reminiscent of that of CRP. Figs 1B and 2 show an example of deposition of C3d in infarcted myocardium at different magnifications. The staining patterns of C3c and C4c were similar but less distinct than C3d and C4d. Remarkably, C4c and C3c deposition was more pronounced in infarctions of greater age (Table 4). Although this may indicate that most of the C4

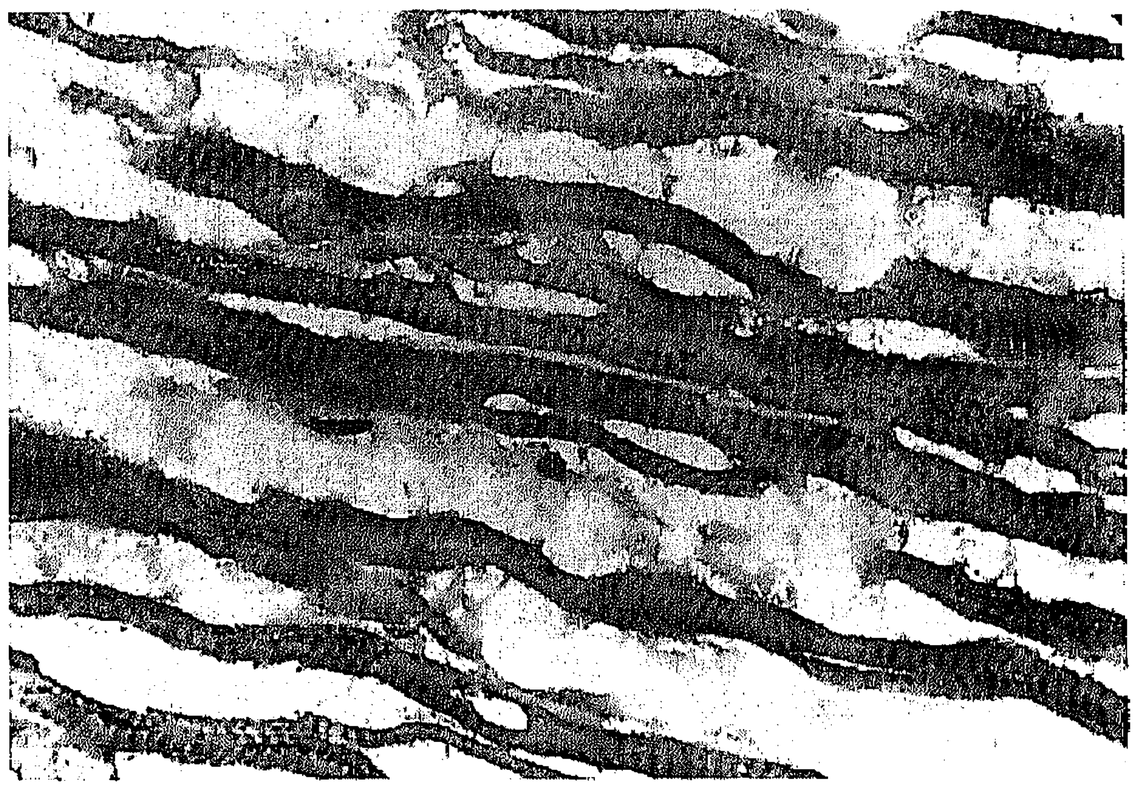

FIG 2. Positive staining for myofiber elements (sarcolemma, cytoplasm, and cross-striation) in infarcted myocardium with MAb C3-15 can be recognized in longitudinally cut myofibers (magnification ×630) (patient 13, Table 2).

and C3 found at the site of complement activation was in the form of C4d and C3d, we cannot exclude the possibility that these observations reflected differences in the efficiency of the antibodies to detect each antigen in that more C4b or C3b deposition was required to obtain a positive staining for C4c or C3c than for C4d or C3d, respectively. Another plausible explanation for these observations, however, might be that only part of the C4 and C3 deposited consisted of C4b/C4bi and C3b/C3bi, respectively, whereas the majority, in particular in younger infarctions, consisted of C4d and C3d. As with CRP, complement depositions were not found in the myocardial samples from 1 patient who died within 12 hours after AMI symptoms had begun, nor were they detected in myocardial samples from 2 patients at the site of previous AMIs from 1 and 10 years previously. In 1 patient with an

TABLE 4. Intensity of Immunostaining of CRP and Complement in Myocardial Infarction

	Infarct Age						
	Hours (No. of Patients)		Days (No. of Patients)				
Immunostaining Antigen	0-12 (2)	12-24 (1)	1-3 (3)	3-5 (3)	5-9 (5)	9-14 (3)	Total (17)
CRP							
Myofiber	1/1/0	0/0/1	0/1/2	0/0/3	0/2/3	0/0/3	1/4/12
Blood vessel	2/0/0	1/0/0	3/0/0	2/0/1	4/0/1	1/1/1	13/1/3
C4d							
Myofiber	2/0/0	0/0/1	0/0/3	0/1/2	0/0/5	0/1/2	2/2/13
Blood vessel	1/1/0	0/0/1	1/1/1	1/0/2	2/1/2	0/1/2	5/4/8
C4c							
Myofiber	2/0/0	0/0/1	2/1/0	1/0/2	1/1/3	0/0/3	6/2/9
Blood vessel	2/0/0	1/0/0	3/0/0	2/0/1	4/0/1	0/1/2	12/1/4
C3d							
Myofiber	1/1/0	0/0/1	0/0/3	0/0/3	0/1/4	0/0/3	1/2/14
Blood vessel	1/1/0	0/0/1	0/0/3	0/0/3	1/0/4	0/0/3	2/1/14
C3c							
Myofiber	1/1/0	0/0/1	0/2/0*	1/1/1	0/0/4*	0/1/2	2/5/8
Blood vessel	2/0/0	0/1/0	2/0/0*	2/1/0	3/1/0*	0/1/2	9/4/2
C3a							
Myofiber	2/0/0	1/0/0	3/0/0	3/0/0	5/0/0	3/0/0	17/0/0
Blood vessel	2/0/0	1/0/0	3/0/0	3/0/0	5/0/0	3/0/0	17/0/0

Values are number of negative immunostaining/number of moderate but positive immunostaining/number of strong, positive immunostaining.

*One slide neglected because of high background staining invalidating proper immunohistochemical scoring procedure.

AMI 0 to 12 hours old, only moderate but significant staining patterns for complement were found. In concordance with the observations for CRP, the complement staining was less distinct than that seen in the older infarct samples. In the infarctions, activated C4 and C3 appeared to be localized in the same areas that had fixed CRP (Fig 1A and 1B). However, these complement fragments were also found to be fixed to blood-vessel elements (endothelium, intima, tunica media, and adventitia; Table 3). Positive cytoplasmic staining for complement was evident in the older infarct samples (1 to 14 days old) but was not as distinct as that observed for CRP (Fig 2). Significant staining also seemed to be independent of the presence of PMN and/or lymphocyte infiltration.

With respect to deposition of complement, we also included a number of controls. First, as with CRP, healthy-appearing areas within the infarcted myocardium did not stain for complement (see Fig 1B). Second, irrelevant MAbs (2 IgG1 and 1 IgG2a), tested at concentrations similar to those for the anti-complement MAbs, yielded negative results. An MAb against C3a (C3-5) did not stain any of the tissue samples from either infarcted or normal myocardium (Tables 3 and 4). This was expected, because C3a, once formed, is released from C3 and will not remain bound to the activator—in our study, myocardial anatomic structures. Finally, the myocardial tissue specimens from the septic patient and the immature child described above did not depict any staining for complement.

Colocalization of CRP and Complement

To test colocalization of CRP and activated complement in infarcted myocardium, we performed double-staining experiments according to the procedure outlined in "Methods." Fig 3 depicts an example of these experiments showing a double immunostaining for CRP (brown) and C3d (blue) of infarcted myocardium. The double staining of CRP and C4d showed similar patterns. In general, CRP was found to be colocalized with activated C4 and C3 in the tissue samples, although in some areas (in particular in the cytoplasm of jeopardized cardiomyocytes in infarction that existed longer than 24 hours), CRP apparently was present in larger quantity, as was evident from the slightly predominating brown color.

FIG 3. Colocalization of CRP and C3d in infarcted myocardium (abbreviations as in Fig 1) shown by immunohistochemical double staining using MAbs 5G4 (brown) and C3-15 (blue) (magnification ×200) (patient 3, Table 2).

Discussion

Hill and Ward[29] were the first to show that complement is activated locally by infarcted myocardium. These results, observed in rats, have been confirmed by a number of other studies in various animal models for AMI.[1,2,22,23] In addition, these studies showed that complement activation in infarcted myocardium occurs via the classic pathway[30,31] and that inhibition of this activation attenuates infiltration of the jeopardized myocardium by neutrophils and reduces infarct size.[1-3,32,33] Thus, complement activation is a key event mediating the deleterious effects of the local inflammatory response occurring in the infarcted myocardium. Our results indicate that complement is activated in human AMI as well, which agrees with previous studies.[22,23] The nature of the substance of the infarcted myocardium that starts activation of complement by binding and activating the first component of complement is unknown. Some studies suggest that constituents of mitochondrial membranes may act as activators.[30,31,34] However, our results indicate that in human AMI, CRP, an acute-phase protein well known for its ability to activate the classic complement pathway in vitro,[13-15] is involved as an activator. Yet, we cannot definitely exclude that other substances able to activate complement are generated in the infarcted myocardium as well.

The major anatomic structure in the infarcted myocardium that had bound CRP (and complement) was the heart muscle cell itself, more specifically the sarcolemma, cytoplasm, and cross-striation, in addition to blood-vessel elements (Figs 1A, 1B, and 2; Tables 3 and 4). Furthermore, positive staining for CRP seemed to be independent of the presence of PMN infiltration in the myocardium. This localization pattern of CRP is comparable to that described in 1963 by Kushner and coworkers,[20] using polyclonal antibodies in infarcted myocardium of rabbits. In other (experimental) inflammatory diseases, CRP has also been found deposited at sites of inflammation.[17,18] Turnover studies with labeled CRP in humans with inflammatory disease, however, have failed to demonstrate localization of this acute-phase protein.[35] Thus, the fraction of the total amount of CRP that localizes in inflamed tissues is apparently small. Our results do not allow conclusions regarding the nature of the ligand for CRP in the infarcted myocardium. However, considering that CRP can bind to vesicles consisting of phosphatidyl choline and lysophosphatidylcholine[13-15] and that on infarction a significant amount of lysophospholipids (which are not detectable in healthy myocardium) are generated in the myocardium,[36] we consider lysophospholipids likely candidates to serve as ligands for CRP.

Involvement of CRP in the local activation of complement was implied by the observation that fixation of CRP and deposition of complement in the infarcted myocardium occurred in an almost identical fashion (Fig 1A and 1B). Double immunostaining techniques (Fig 3) indeed confirmed that CRP and activated complement for the major part were localized at similar anatomic sites.

Previously we have shown that MAb C4-1 and MAb C3-9 recognize activated C4 and C3, respectively, and not native C4 or C3[25,26] and that a similar specificity holds for immunohistochemical applications.[37] Therefore, positive immunostaining with these MAbs ruled out the possibility that the results were influenced by aspecific binding of C4 and C3 to the infarcted myocardium. However, the epitopes recognized by MAb C4-1 and MAb C3-9 are also expressed on C3 with a cleaved thioester,[25,26] implying that

positive immunostaining with these MAbs may have been due to nonspecific binding of native C4 and C3 to the infarcted tissue with subsequent cleavage of the thioester, for example due to fixation procedures or storage conditions of the tissue samples. However, this possibility could be ruled out by the negative staining with MAb C3-5, which recognizes the C3a part of C3 with a cleaved thioester.[25] Therefore, the depositions of C4 and C3 in the infarcted myocardium reflected complement activation and not nonspecific binding, in concordance with conclusions reached by others.[23]

Remarkably, although vascular elements clearly appeared to have bound complement activation products (C3b/bi/d and C4b/bi/d), they contained only small amounts of CRP, if any. Endothelial cells can synthesize complement proteins in vitro.[33] However, the endothelial deposition observed in the patients described here probably did not reflect synthesis of complement proteins by endothelial cells, because two of the MAbs used (C4-1 and C3-9) are specific for activated C4 or C3, respectively, hardly recognizing the native proteins.[25,26] Furthermore, the C3 species fixed to the endothelium did not contain detectable C3a, consistent with the proposal that this C3 species represented activated C3 and not native C3. The presence of activated complement components on endothelial structures in the absence of CRP may indicate that molecules other than CRP are involved in activating complement in infarcted myocardium. However, it cannot be excluded that CRP had dissociated from the endothelial structures after having activated complement. In agreement with this is the observation that patients with AMI have increasing plasma levels of CRP complexed to activated C3 or C4 (W.K.L., C.A.V., W.T.H., G.J.W., C.E.H., unpublished observations). Alternatively, the sensitivity of detecting activated complement by immunohistochemistry may have been superior to that of detecting CRP. The presence of moderate amounts of CRP bound to endothelial structures in some tissue specimens supports this latter notion.

Fixation of CRP or complement was not or was only very moderately seen in the tissue samples of a very recent infarction (<12 hours earlier) or those of old infarction (>1 year earlier). Thus, it is tempting to speculate that CRP fixation (as well as complement deposition) in human AMI starts within 12 hours, lasts beyond 14 days, and decreases with time thereafter. CRP plasma concentrations start to rise ≈8 to 10 hours after challenge with the inciting stimulus,[38] for example, myocardial infarction.[5,7,8] The absence of CRP in early infarctions (0 to 12 hours old), therefore, is not surprising. Moreover, the absence of complement in the early infarctions, which has also been noted in animal models,[22,23] supports the theory that CRP is a main activator of complement during the early phases (12 to 24 hours) of AMI. In agreement, the intensity of the staining patterns of CRP and complement in the tissue samples of the infarctions both tended to increase with the age of the infarction, being optimal at 3 days. These kinetics of CRP deposition in the myocardium parallel the systemic response of CRP levels, which reach a peak after ≈72 hours and decline thereafter.[5] It is to be noted that an increase of circulating CRP itself was not sufficient to yield localization of CRP in the myocardium, because in a patient with sepsis we did not detect CRP in the myocardium and the healthy-appearing areas of the infarcted myocardium did not contain detectable CRP. Altogether, our data predict that interventions at the level of CRP or complement should be applied within 12 hours up to 3 days after start of AMI, at least in patients not receiving reperfusion therapy.

In conclusion, we show localization of CRP in infarcted myocardium. We suggest that CRP, by activating complement, enhances inflammation and hence promotes tissue damage in AMI. Moreover, we think that our observations might have pathophysiological implications beyond myocardial infarction, because in other inflammatory diseases (eg, rheumatoid arthritis, inflammatory bowel disease, sepsis, and meningitis), similar CRP and complement responses are seen. Future studies are warranted to investigate whether this proinflammatory role of CRP may lead to new therapeutic approaches in AMI and in other inflammatory diseases.

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